

## Determination of Allele Frequency of the CD18 gene in Animals Girolando

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The Bovine Leukocyte Adhesion Deficiency (BLAD) is an inherited autosomal recessive character caused by two point mutations in the *CD18* gene. One occurs in the nucleotide 383 with the exchange of an adenine by a guanine leading to the substitution of a glycine by an aspartic acid. The other mutation occurs in the nucleotide 775 through an exchange of cytosine by thymine. The objective of the present work is to estimate the genotypic and allelic frequencies of CD18 gene on Girolando breed cattle and verify if the population is under Hardy-Weinberg equilibrium (HWE). Eight hundred eight two animal, participants on Girolando Progeny Test, coordinated by Embrapa Dairy Cattle and Brazilian Association of Breeders, were genotyped. DNA was extracted from blood and semen samples using DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany), quantified and evaluated by spectrophotometry (Nanodrop®, Wilmington, DE, USA). The identification of carriers was realized using PCR-RFLP technique. The amplified product was digested with *Hae III* and *Taq I* restriction enzyme (Promega Corporation, Foster City, CA, USA), and restriction fragments were observed on 3.5% agarose gel dyed with ethidium bromide. Allelic and genotypic frequencies and HWE were established using POPGENE software (v 1.32) and the probability of HWE was tested using  $\chi^2$  ( $p < 0.05$ ). The establishment of the phenotype was done as follows: in carrier animal (BL) with *Taq I* enzyme the bands showed were 58 bp, 32 bp and 26 bp, and with *Hae III* enzyme the bands showed were 49 bp, 30 bp, 19 bp and 9 bp. For non-carriers (TL) of enzymes *Taq I* and *Hae III* were identified the bands 32 bp, 26 bp, 49 bp and 9 bp. The frequency of non-carriers animals was 99.32% and carriers animals was 0.67% and the allelic frequencies was 99.66% for the allele T and 0.34% for allele B. The result obtained in this study corroborates the findings of Ribeiro et al. (2000) and Riojas-Valdes et al. (2009) in studies conducted with animals of Holstein breed. The observed frequencies are close to the expected, indicating that the population is in HWE. Financial Support: CNPq, CAPES.